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Attorney's Docket No. 5470-238

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

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In re: MacDonald et al.
Serial No. 09/288,837
Filed: 8 April 1999
For: *METHODS AND MODIFIED CELLS FOR THE TREATMENT OF CANCER*

Group Art Unit: 1642
Examiner: B. Brumback

Commissioner for Patents
Washington, DC 20231

Declaration of Robert A. Olmsted, Ph.D.

Pursuant to 37 C.F.R. § 1.132

I, Robert A. Olmsted, do hereby declare and state as follows:

1. I am Vice President of Research at AlphaVax, Inc., the exclusive licensee of U.S. Application Serial Number 09/288,837 (*hereinafter*, "the '837 application").

2. I commissioned a study on the use of alphavirus replicon particles expressing a cancer antigen to immunize mice with the goal of preventing tumor formation or reducing tumor size following challenge with breast tumor cells. My laboratory produced the alphavirus replicon vector particles (hereinafter "VRPs"), generally according to the methods described in Example 1 of the '837 application, namely a VEE replicon RNA expression vector encoding a cancer antigen, and two helper RNA vectors encoding the VEE 3014 structural proteins, that together when introduced into a packaging cell, produce VRPs. The cancer antigen is the rat *neu* oncogene (P185), GeneBank accession #X03362 (Bargmann et al., (1986) *Nature* 319: 226-230). The investigators performing the study were instructed to vaccinate mice three times (at Day 0, 14 and 21) in one foot pad, at the following doses: 1 x 10^6 VRP expressing HA (negative control), 1 x 10^5 VRP expressing the rat *neu* gene, and 1 x 10^6 VRP expressing the rat *neu* gene.

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3. The results of the study can be summarized as follows: There were 8 mice in each treatment group (one negative control group and two treatment groups at different VRP doses). The mice were immunized on Days 0, 14 and 21 of the experiment. Following vaccination (Day 35), each group was injected with tumor

cells in the mammary fat pad. The tumor cells were developed from a tumorigenic and metastatic mammary tumor in Balb/c mice. A cell line was established from this tumor by stably transforming these cells with the her2/neu gene, and cells of this line were injected into the mice. Tumor development was measured at 14 days post-tumor cell challenge. In the HA-VRP group (the control group), 7/8 mice developed tumors. In the treatment groups, only one mouse in the 1 x 10^6 dose group developed tumors, and none of the mice in the 1 x 10^5 group had tumors.

A FACS analysis of antisera pooled from mice in each group was performed using the A2L2 cell line as the target (this cell line, expressing the rat *neu* gene, is the cell line used as the tumor challenge; see Lachman et al., (2001) *Cancer Gene Therapy* 8: 259-268). Following three vaccinations, all of the mice from the two VRP-*neu* treatment groups were strongly positive at three serial dilutions, while the control mice were negative. Even after only a single dose of the VRP-*neu* at 1 x 10^5, a strong antibody response could be detected.

4. From these results, it is clear that VRPs expressing the rat *neu* gene are protective against tumor induction in this mouse model system.

5. I do hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 19 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Robert A. Olmsted, Ph.D. Date July 10, 2001

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